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MEHP/DEHP: Gonadal Toxicity and Effects on Rodent Accessory Sex Organs

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The phthalate acid esters (PAEs), and, in particular, di(2-ethylhexyl) phthalate (DEHP) and its monoester, monoethylhexyl phthalate (MEHP), can adversely affect rodent testes but only at high doses. Rat gonadal zinc levels can be decreased by the injection of DEHP, but not MEHP. The rat prostate gland seems to be particularly sensitive to PAE-induced zinc depletion. PAE-induced changes in male reproductive organs were more evident in the rat than in the mouse. Some of the effects of MEHP can be demonstrated *in vitro* since it can alter the uptake of ^{65}Zn in rodent gonads and accessory sex organs.

Introduction

There has been recently renewed interest in the gonadal toxicity exerted by certain of the phthalate acid esters (PAEs). After some early observations by Shaffer et al. (*1*) reporting that diethylhexyl phthalate (DEHP) could adversely affect rodent testes, little attention was directed toward such actions for nearly 30 years. In 1977, Gray et al. (*2*) examined the short-term toxic effects of DEHP in rats and reported that testicular weights were significantly decreased at higher dietary levels of this PAE. More recently, and examining one of the principle metabolites of DEHP, namely, mono-ethylhexyl phthalate (MEHP), Oishi and Hiraga (*3-6*) have begun to shed some light upon certain aspects of gonadal toxicity. Dietary DEHP or MEHP can cause severe testicular damage in rats (*3*, *4*). Comparable damage was recorded in mouse testes (*5*, *6*). These investigators also reported that zinc levels in testes obtained from rats previously injected with MEHP were reduced (*4*). Other phthalates can alter zinc excretion in rats (*7*). Gonadal testosterone levels appear to remain unchanged by MEHP (*4*), but DEHP seems to lower the secretion of testosterone in the rat (*8*).

Many of the toxicologic effects upon either the male or female reproductive system have been recently reviewed (*9*). Some of the more major actions of MEHP and/or DEHP upon the male and female reproductive system have been summarized on Table 1. Other speakers at this conference will deal more specifically with the effects of phthalates on the female reproductive system and upon its feto- or embryotoxicity. Previous studies from this laboratory reported that MEHP was not teratogenic in rabbits (*15*).

The present experiments were undertaken to further investigate the actions of MEHP and DEHP upon male reproductive organs of the sexually mature rat and mouse. Gonadal and accessory sex organ zinc and nucleic acids were examined in rodents previously treated with either of these phthalates. Efforts were made to evaluate the effects of these two phthalates in relation to dose, route of administration and whether or not PAE-induced actions could be elucidated *in vitro*.

Methods

Male mice (Swiss-Webster) and rats (Sprague-Dawley) with average body weights of 37 g and 250 g, respectively, were used in these investigations.

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None of the doses of phthalates used in these experiments caused any impairment of body weight.

DEHP was obtained commercially (ICN Pharmaceuticals, Plainview, N. J.), while MEHP was generously supplied by Travenol Laboratories (Deerfield, Ill.). Appropriate vehicle-treated controls were run concurrently and consisted of 0.9% NaCl for MEHP and peanut oil for DEHP-treated animals. Routes of administration were either subcutaneous or intraperitoneal with animals being sacrificed 24 hr after the final injection. Various *in vivo* and *in vitro* doses or concentrations were employed depending upon the experimental design.

Organs were analyzed for endogenous zinc by atomic absorption spectrophotometry (Perkin-Elmer 305B) after initially digesting tissues in concentrated nitric acid overnight. Some reproductive system organs were analyzed spectrophotometrically for DNA (*16*) and also for RNA levels (*17*). The *in vitro* experiments utilized tissue slices (approximately 30 mg) bathed in an incubating medium of Krebs-Ringer bicarbonate buffer (pH 7.4) containing MEHP (5 or 10 *nM*) and ^{65}Zn (0.01 μCi/ml). Incubations were carried out at 37°C for periods of 1 or 2 hr in a Dubnoff metabolic shaker. Tissue levels of ^{65}Zn were subsequently analyzed on a gamma counter and were corrected for decay and background. Statistical analyses were carried out where appropriate.

Results

Table 2 depicts the action(s) of either MEHP or DEHP upon mouse endogenous zinc and nucleic acids. Neither dose nor phthalate affected testicular zinc or DNA/RNA ratios. There was some evidence of MEHP toxicity at the highest dose, namely, 100 mg/kg, as evidenced by a 50% mortality (Table 2).

Extending the duration of administration to an overall period of 20 days led to some significant changes in certain reproductive organs (Table 3).

Table 1. Effects of phthalate acid esters (PAEs) on the male reproductive system.

Species	PAE	Effect	Reference
Rat	DEHP	Testicular degeneration	Shaffer et al. (*1*)
Rat	DOP	Decreased testes weight	Harris, (*10*)
Rat	DEHP	Early fetal death and semisterility	Singh et al. (*11*)
Ferret	DEHP	Testicular degeneration	Lake et al. (*12*)
Rat	DEHP	Testis histological damage	Gray (*2*)
Rat	DEHP	↓ Testes weight ↑ Testosterone ↓ Zinc	Oishi and Hiraga (*8*)
Rat	DEHP	Testicular atrophy	Foster (*7*)
Mouse	DEHP	↓ Testosterone ↑ Testes weight ↓ Zinc	Oishi and Hiraga (*3*)
Rat	DEHP	↓ Testes weight ↑ Testosterone ↓ Zinc	Oishi and Hiraga (*4*)
Rat	DMP	↓ Testes weight ↑ Zinc excretion	Cater (*13*)
Rat	MEHP, MBP, MIBP	Testicular atrophy ↓ Zinc ↑ Testosterone	Oishi and Hiraga (*5*)
Mouse	MEHP, MBP, MIBP	↓ Testosterone ↑ Testicular weight	Oishi and Hiraga (*6*)
Rat	DEHP, DA79P	↓ Testicular weight	Mangham et al. (*14*)

Table 2. Action of MEHP or DEHP (50, 100 mg/kg daily × 5, IP) on mouse testes.

Ester	Concn.	n^a	Zinc, % of control	DNA/RNA ratio
MEHP	Control	6	—	+3.2
	50 mg/kg	6	–1	+2.7
	100 mg/kg	3^b	–22	+3.0
DEHP	Control	6	—	+2.4
	50 mg/kg	6	0	+2.3
	100 mg/kg	6	–1	–2.7

[a]Number of animals/group.
[b]Of the six mice injected, three died.

Table 3. Gonadal responses of mice and rats to MEHP and/or DEHP.[a]

			Anterior prostate, % of control		Testes, % of control	
Ester	n[b]	Species	Weight	Zinc	Weight	Zinc
MEHP, 50 mg/kg	6	Mice	+3	+1	-3	+1
DEHP, 100 mg/kg	6	Mice	+9	-11	+7	-3
MEHP, 50 mg/kg	6	Rats	-18	-37[c]	-6	+9
DEHP, 100 mg/kg	5	Rats	-3	-34[c]	-6	-31[c]

[a]Animals were injected (IP) every other day for a period of 20 days.
[b]Number of animals/group.
[c]Significantly different from control ($p \leq 0.05$).

Table 4. Effect of previously injected MEHP (50 mg/kg, IP, every other day for 20 days) on the *in vitro* assimilation of ^{65}Zn by mouse reproductive organs.

			^{65}Zn uptake interval, % of control	
	n[a]	Organ	1 hr	2 hr
Control	6	Testes	—	—
MEHP	6		+11	-34
Control	6	Anterior prostate	—	—
MEHP	6		+75	+149[b]

[a]Number of slices.
[b]Significant at $p \leq 0.05$.

Table 5. Action of MEHP on the uptake of ^{65}Zn by slices of rat testes and ventral prostate *in vitro*.

MEHP level, *mM*	n[a]	Uptake, % of control[b]	
		Testes	Prostate
5	8	+22	+57[c]
10	10	+108[c]	+74[c]

[a]Number of slices.
[b]Uptake at 1 hr.
[c]Significant ($p \leq 0.05$).

Such changes, however, were only seen in the rat. Comparable doses of either MEHP or DEHP failed to modify testicular zinc or prostate zinc in the mouse. It is interesting to note that while zinc concentrations were significantly reduced (p 0.05), gravimetric responses remained unchanged in the rat gonad.

In an effort to examine further the lack of sensitivity of the mouse to PAE-induced changes in reproductive organs, a series of *in vitro* experiments were undertaken. Table 4 reveals that when mice were injected with MEHP and the organs subsequently examined for their ability to assimilate ^{65}Zn *in vitro*, that the prostates from the PAE-treated showed significantly higher levels of radioactivity. These prostate effects appeared to be time-related whereas no pattern of response was forthcoming in the mouse testes (Table 4).

The actions of MEHP *in vitro* were studied in the rat testes and prostate gland (Table 5). Using this *in vitro* experimental design rat zinc was again observed to be altered by MEHP. Both *in vitro* concentrations appeared to be effective in enhancing the assimilation of ^{65}Zn. The rat ventral prostate was somewhat more sensitive to MEHP-induced changes in ^{65}Zn assimilation than the testes (Table 5).

Discussion

The present studies, coupled with other recently published studies from this laboratory using rodent testes and prostate (*18*, *19*), serve to confirm some of the previously reported effects of phthalates on the male reproductive system. The present studies, however, reveal several no-effect dose levels. More importantly, and based on the fact that the present studies employed nonoral routes of PAE administration, the action of either MEHP or DEHP in affecting depletion of endogenous gonadal zinc was not due to interference of the intestinal absorption of this divalent ion. Previous studies utilizing dietary MEHP or DEHP could not exclude this possibility as a mechanism for gonadal toxicity. A number of dietary constituents, including phytate, can interfere with the absorption of zinc (*20*).

The present studies tend to suggest that the mouse reproductive system is somewhat more resistant to the effects of the phthalates than the rat. The reason for this difference in gonadotoxicity is not evident, but it does not appear to be due to lower endogenous levels of testicular zinc in the rat.

The prostate gland, regardless of species, seems to be more profoundly affected by phthalates than does the gonad. Endogenous zinc can be depleted by both MEHP and DEHP in the rat ventral prostate. Only DEHP caused a slight reduction in endogenous zinc in the anterior lobe of the mouse prostate. However, the assimilation of ^{65}Zn by the mouse prostate and by the rat prostate was particularly enhanced by MEHP. Among the various zinc-containing organs in the mammal, the prostate reportedly contains relatively high levels of this ion (*21*). Zinc, along with a number of other divalent ions, can interfere with the binding of dihydrotestosterone to its receptor(s) in mouse prostate gland (*22*).

Exactly how the phthalates facilitate the turnover of endogenous zinc remains to be established. Phthalates can markedly increase the urinary excretion of zinc. It is possible that in the present studies that MEHP or DEHP facilitated the turnover of endogenous zinc thereby allowing increased incorporation of ^{65}Zn into the prostate or testes. Whatever the mechanism of phthalate-induced modifications in zinc metabolism, the present studies reveal that MEHP or DEHP can exert such actions both *in vivo* and *in vitro*.

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